mature endocrine cell numbers, as well as gradual loss of islet cell mass. MafA and Arx, key islet cell gene products, were also found to be Isl1 transcriptional targets (Diabetes. 2009 Sep; 58(9):2059–69 and J Biol Chem. 2011 Mar 9). The LIM domains of Isl1 can function as protein interaction interfaces for other transcription factors and co-regulators. In this study, we investigated the impact of the LIM protein co-factor, Ldb1, in endocrine pancreas formation. Ldb1 expression was detected widely during early embryogenesis in the pancreatic buds and the surrounding mesenchyme, but later became highly enriched in islets and pancreatic ducts. Loss of Ldb1 in Pax6 (Le)-Cre;Ldb1Fl/Fl mice was found to cause an overt reduction in insulin+ and glucagon+ cell numbers by E18.5. The remaining insulin+ cells appear dysfunctional, as they lacked MafA and Glut2 in beta cells. Ldb1-deficient animals also suffered from overt diabetes due to greatly reduced islet cell mass. Presently, we are examining by microarray and ChIP-Seq analysis the similarities between Ldb1 and Isl1 regulation of beta cells, with the expectation that differences may be found due to the expression Lmo2 and Lmo4 in the developing pancreas.

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Program/Abstract #463
Vangl2, aPKC and VAMP1; the interactions of polarity proteins with trafficking vesicle proteins in the *Xenopus* oocyte
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The *Xenopus* oocyte is a polarized cell with a distinct animal/vegetal axis. It contains the components of both PCP and apical /basal polarity systems, including Vangl2 and flamingo (planar cell polarity proteins) and aPKC, PAR3 and 6 (apical basal polarity proteins). To begin to address the relative roles of these systems in the oocyte, we examined the localization and function of Vangl2 and aPKC. We found that: 1. Vangl2 is enriched in radially arranged islands in the animal hemisphere. 2. Vangl2 co-localizes and physically interacts with VAMP1, a component of post-Golgi membrane trafficking vesicles. 3. The localization of animally localized VAMP islands depends upon Vangl2 protein. 4. The arrangement of VAMP1/Vangl2 complexes depends on the stable acetylated microtubule cytoskeleton 5. aPKC also physically interacts with Vangl2 6. aPKC is required for the stability of the radially arranged acetylated microtubule cytoskeleton and the location of VAMP1/Vangl2 complexes. We conclude that both maternal aPKC and Vangl2 are essential for the polarity of the *Xenopus* oocyte.

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Program/Abstract #464
A novel role for a Cdc42 effector protein in *Xenopus* neurogenesis
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Many developmental events, such as axis determination and cell movement, are dependent on the asymmetrical distribution of proteins and RNAs. In *Xenopus laevis*, several maternal mRNAs essential for normal development are localized to the oocyte vegetal cortex. In this work we characterize a novel cortex-enriched transcript, cdc42 effector protein 4-like (cep4l). CEPs bind cdc42 and related small GTPases, which regulate many cellular functions. cep4l is expressed in the oocyte vegetal cortex and throughout embryonic development, including expression in migratory cells during gastrulation, neural crest in neurulae and tailbud stages, and neural regions in older embryos. Misexpressed cep4l RNA causes convergent extension defects and induces ectopic neuronal marker expression, indicating a role in neurogenesis. Experiments to identify upstream and downstream pathways indicate roles for FGF as well as cdc42. Co-expression studies with another neuronal inducer, FGF8a, demonstrate an enhancement of cdc42 binding and ectopic neurogenesis. The effects of both cep4l and FGF8a are independent of proliferation. We also present loss of function data showing a role for cep4l in normal axial and nervous system development, as well as a requirement for FGF8a-induced neurogenesis. Although the roles of small GTPases in cell division, migration, and adhesion are well-characterized, our results suggest novel roles and pathways for these proteins and their effectors in neural fate patterning and neurogenesis.

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Program/Abstract #465
Expression in dorsal–lateral regions of *Drosophila* early embryos is supported by Grainyhead-mediated anti-repression
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The *Drosophila* pre-gastrula embryo is patterned by a nuclear gradient of the transcription factor Dorsal, which supports expression of genes in defined domains along the dorsal–ventral axis. Current models postulate that limiting amounts of Dorsal establish the dorsal boundaries of gene expression. In the case of the gene intermediate neuroblast defective (ind) which is expressed in a dorsal–lateral stripe, in addition, EGFR signaling also supports ind expression. We have evidence suggesting repressors are necessary for the sharp dorsal border of ind. A synthetic enhancer analysis of the ind enhancer located a short 12 base pair repetitive sequence ("A-box") that mediates transcriptional repression in dorsal regions of embryos (Stathopoulos & Levine, DevBio 2005). We found that this element alone is sufficient to mediate repression in dorsal regions, furthermore, when this element is mutated in the full-length enhancer expression is expanded dorsally. We identified proteins that bind this element using affinity chromatography and mass spectrometry. One of the factors we identified is grainy head (grh), a DNA-binding protein, which surprisingly we found acts as an activator to support expression of ind and has been shown to exhibit context-depedent activation that is influenced by MAPK signaling. We believe that grh acts to inhibit the repressor that sets the dorsal border of ind by competitively binding to the A-box element. Instead of limiting activators being responsible for establishing the dorsal boundary of ind, we propose instead that expression of this gene is supported by the grh activator that functions to limit the effects of repressors.

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Program/Abstract # 466
A high throughout sequencing-based screen for sea urchin skeletal patterning genes
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Pattern formation is a crucial event that occurs at all scales during embryonic development. Sea urchin larvae possess a skeleton which is secreted by the primary mesenchyme cells (PMCs). However, skeletal patterning information is localized within the ectodermal cells, and is detected by thin filopodia extended from the PMCs. SB23580 (SB) and NiCl2 treatments provoke opposite ectodermal effects, since SB dorsalizes, while nickel ventralizes the ectoderm. However, transient exposure to either perturbant induces dramatic skeletal patterning defects via ectodermal perturbation. We predicted that ectodermal patterning genes are absent in both nickel- and SB-treated embryos, and are a minority cohort of co-regulated genes, while the majority of genes are reciprocally regulated by SB and nickel. We therefore used RNA-seq analysis of control, SB-, and nickel-treated embryos to identify genes mutually down-regulated by both perturbants. This group of genes represents 1.5% of scaffolds, compared to ~20% of scaffolds affected by each single perturbant. 72 candidates, corresponding to mutually-downregulated genes that encode surface proteins, were identified. These conserved genes include Reelin, BMP5-8, Notch2, Mindbomb, ST-14, 5-LOX, Prestin, SVEP, MLD, RECK. Functional characterization demonstrates that these candidates are specifically required for skeletal patterning and do not impact ectodermal specification. Morphant phenotypes reveal novel and dramatic skeletal patterning defects which are reflected in defective PMC migration patterns. Thus, patterning of the sea urchin skeleton reflects a functional convergence of genes that, in vertebrates, control diverse processes ranging from neocortical patterning, auditory amplification, vasculogenesis, and metastasis. Interestingly, sea urchin larva lacks a neocortex, a vasculature, audition, and cancer. Thus, sea urchin skeletal patterning provides a surprising and unexpected glimpse of the ancestral functions for this cohort of genes.

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Program/Abstract #467
Identification of the gene responsible for the wings apart phenotype in *Drosophila melanogaster*
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The Drosophila wings apart (wap) locus contains a semi-lethal gene that when mutated leads to the absence of the Tergal Depressor of Trochanter (TDT) muscle. wap has been mapped to the proximal X chromosome but it is unclear what gene is mutated to produce the wap phenotype. The aspect of muscle development disrupted in wap mutants leading to TDT loss is also unknown. To identify the wap gene, we performed complementation mapping of wap mutants crossed with known X chromosome deletions. We sectioned thoraces of progeny from these crosses to observe if these flies exhibit the TDT phenotype associated with wap. Results of mapping analysis and phenotypic characterization suggest the most likely candidate for the wap gene is DIP1. PCR of DIP1 underway in wild-type and wap mutant flies to detect the mutation leading to the observed phenotype has shown an alanine to threonine amino acid substitution in the DIP1 coding region in wap mutants. Loss- and gain-of-function assays are in progress to determine if loss of DIP1 reproduces the wap mutant phenotypes and if over-expression of DIP1 rescues the wild-type phenotype. The impact of the wap mutation will be analyzed by determining at which step in development TDT muscle formation is disrupted. Initial experiments have shown that wap mutants lack TDT specifying founder cells, suggesting wap is necessary for early TDT specification. The broad goal of this research is to identify mechanisms of muscle formation in the Drosophila adult. Since similar developmental mechanisms are used in vertebrate and invertebrate muscle formation, this study can aid in understanding processes which may impact vertebrate muscle formation and whose mis-regulation may lead to muscular diseases.

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Program/Abstract #468
Live imaging of stomatal determinants reveals dynamic interaction among precursor cells
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Stomata, watertight valves on the plant surface, allow land plants to survive in dry conditions by facilitating gas exchange while moderating water loss. Their distribution on the plant epidermis is even and nonrandom; stomata are not found adjacent to one another. The bHLH transcription factors SCREAM (SCRM) and SCRM2 are robustly expressed throughout stomatal development and have been shown to interact with the bHLHs SPEECHLESS, MUTE, and FAMA. These heterodimers specify three sequential cell-state transitions critical to stomatal differentiation. Live time-lapse imaging of a translational fusion of SCRM with green fluorescent protein (GFP) under its native promoter shows that SCRM-expressing cells arise in pairs on a germinating cotyledon. As a rule, one cell differentiates into a stoma, while one divides asymmetrically away from it, maintaining the one-cell spacing rule. Computational analysis of fluorescence intensity determines the relationship of GFP–SCRM expression level to cell fate in unperturbed cotyledons, signaling mutant backgrounds, and cell ablation contexts leading to possible cell fate change.

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